# METHODS FOR PRODUCING A VEGETABLE PRODUCT

## Field of the Invention

The present invention relates to methods for producing a vacuum packed pre-boiled vegetable product.

## Background

Vacuum packed pre-boiled vegetable products are widely used by the food service sector, catering, institutions as well as by private households.

A conventional process for production for a vacuum packed pre-boiled vegetable product, such as a vacuum packed pre-boiled potato product, may comprise the steps of washing, peeling, cutting, packaging, boiling and cooling. The vacuum packed pre-boiled vegetable product may be stored refrigerated at approximately 4°C, for up to several weeks. During storage an off-flavour/off-taste may develop as a result of oxidative processes. It is an object of the present disclosure to provide improved methods for producing a vacuum packed pre-boiled vegetable product comprising vegetables or vegetable pieces having an improved flavour/taste when the package is opened and prepared for consumption following storage.

## Summary of the Invention

The present invention relates to a method for producing from a vegetable a vacuum packed pre-boiled vegetable product, comprising: a) removing the peel from the vegetable, b) contacting the vegetable with an effective amount of an oxidoreductase enzyme; and, c) vacuum packaging the enzyme-treated vegetable, wherein the enzyme-treated vegetable are boiled before or after step (c) to produce a vacuum packed pre-boiled vegetable product.

The present invention further relates to a method for packaging a vegetable product, comprising: a) adding to the vegetable an effective amount of an oxidoreductase enzyme; and, b) vacuum packaging the enzyme-treated vegetable, wherein the enzyme-treated vegetable are boiled before or after step (b) to produce a vacuum packed pre-boiled vegetable product.

The invention also relates to vacuum packed pre-boiled vegetable products obtained by the methods of the present invention.

## Detailed Description of the Invention

The vacuum packed pre-boiled vegetable product of the present invention may be produced from any edible vegetable, preferred are root vegetables, i.e. any fleshy edible underground roots or tubers, e.g. beet, beetroot, cassava, celeriac, celery root, cocoyam,

dasheen, edda, Irish potato, Jerusalem artichoke, murphy, parsnip, potato, radish, salsify, sunchoke, sweet potato, taro, taro root, tater, turnip, vegetable, veggie, white potato, yam. Preferred for the invention are potato and carrot.

In the methods of the present invention, the potato may by of any variety and/or cultivar including, but not limited to Agata, Agria, Alex, Amadeus, Arno, Artana, Asparges, Asva, Atlantic, Balanse, Berber, Bintje, Burren, Calla, Carrera, Centennial Russet, Dali, Danva, Desiree, Ditta, Exempla, Exquisa, Fakse, Filea, Folva, Fontane ,Godiva, Green Mountain, Hamlet, Hanna, Hansa, Hela, Imperia, Inova, Jaerla, Jutlandia, Kardal, Kardent, Karida, Karnico, Kennebec, Kenva, King Edward, Kuras, Lady Rosetta, Laura, Liva, Marabel, Marion, Mercury, Milva Revelino, Minea, Nicola, Norchip, Norgold Russet "BC", Norland, Octavia, Oleva, Panda, Posmo, Primula, Producent, Raja, Raja Bonanza, Red Pontiac, Red Warba, Revelino, Russet Burbank , Sava, Sebago, Secura, Senator, Seresta, Shepody, Sibu, Sieglinde, Sirtema, Stefano, Superior, Sydens Dronning, Symfonia, Tertus, Timate, Tivoli, Torva, Ukama, Victoria, Vivaldi, and White Rose.

The vegetable may be peeled using any appropriate method, e.g. by steam peeling, or by applying an alkali solution followed by mechanical action, e.g. brushing. Apart from being peeled the vegetable may remain whole or it may be divided into smaller parts before being packaged, such as by slicing, chopping or by another appropriate technique to produce vegetable pieces, e.g. in the shape of slices or strips.

As described a vacuum packed pre-boiled vegetable product may develop an off-flavour/off-taste during storage. This off-flavour/off-taste may be due to oxidative processes. By contacting the vegetable/vegetable pieces with an enzyme composition comprising an oxidoreductase enzyme the off-flavour/off-taste can be reduced. Without being bound by theory it is proposed that the beneficial effect is due to the action of the enzyme composition reducing the amount of oxygen present on the surface of the vegetable/vegetable pieces and in the residual process water accompanying the vacuum packaged vegetable/vegetable pieces. Oxidative processes which would otherwise give rise to volatile compounds coursing off-flavour/off-taste are thereby inhibited.

The oxidoreductase enzyme may be any oxidoreductase enzyme selected from the list consisting of; glucose oxidase, galactose oxidase, hexose oxidase, carbohydrate oxidase, pyranose oxidase, amino acid oxidase, and laccase. Preferably the oxidoreductase enzyme is a glucose oxidase. In an especially preferred embodiment the enzyme composition further comprises a catalase.

Glucose oxidase (EC 1.1.3.4) catalyzes the reaction beta-D-glucose + $O_2$ <=> D-glucono-1,5-lactone + $H_2O_2$. Catalase (EC 1.11.1.6) catalyzes the reaction $H_2O_2$ <=> ½ $O_2$ + $H_2O$. The over-all result is the consummation of ½$O_2$ for every molecule of glucose oxidized.

Glucose may be added as a substrate for the oxidoreductase, particularly in the amount of 0.005% to 10%, more particularly 0.01 to 2.5% (w/w), or most particular in the

amount of 0.05 to 0.5 % (w/w). In an embodiment the vegetable/vegetable pieces are further contacted with an additional enzyme, such as a pectinase, an alpha-amylase, an amyloglucosidase or a maltogenic alpha-amylase.

In the methods of the present invention, any oxidoreductase and/or glucose oxidase may be used which possesses suitable enzyme activity in an appropriate pH and temperature range. It is preferable that the enzymes are active over broad pH and temperature ranges.

In a preferred embodiment, the enzymes have a pH optimum in the range of about 3 to about 10. In a more preferred embodiment, the enzyme(s) has a pH optimum in the range of about 4.5 to about 8.5.

In another preferred embodiment, the enzymes have a temperature optimum in the range of about 5°C to about 100°C. In a more preferred embodiment, the enzymes have a temperature optimum in the range of about 25°C to about 75°C.

The term "effective amount" is defined herein as an amount of one or more enzymes that is sufficient for providing a measurable effect on at least one property of interest of the vegetable product.

The term "property of interest" is defined herein as the flavour and/or taste qualities of the vegetable product.

The source of the enzymes is not critical for use in the methods of the present invention for improving one or more properties of a vegetable product. Accordingly, the enzymes may be obtained from any source such as a plant, micro organism, or animal. The enzymes are preferably obtained from a microbial source, such as a bacterium or a fungus, e.g., a filamentous fungus or yeast and may be obtained by techniques conventionally used in the art.

In a preferred embodiment, the enzymes are obtained from a bacterial source. For example, the enzymes may be obtained from an *Acetobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Azotobacter, Bacillus, Comamonas, Clostridium, Gluconobacter, Halobacterium, Mycobacterium, Rhizobium, Salmonella, Serratia, Streptomyces, E. coli, Pseudomonas, Wolinella,* or *methylotrophic* bacterium strain.

In a more preferred embodiment, the enzymes are obtained from an *Acetobacter aceti, Alcaligenes faecalis, Arthrobacter oxidans, Azotobacter vinelandii, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus anitratum, Bacillus brevis, Bacillus circulans, Bacillus coagulans, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus stearothermophilus, Bacillus subtilis, Bacillus thuringiensis, Comamonas testosteroni, Clostridum tyrobutyricum, Gluconobacter dioxyaceticus, Gluconobacter liquefaciens, Gluconobacter suboxydans, Halobacterium cutirubrum, Mycobacterium convolutum, Rhizobium melioti, Salmonella typhimurium, Serratia marcescens, Streptomyces lividans, Streptomyces murinus, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida,* or *Wolinella succinogens* strain.

In another preferred embodiment, the enzymes are obtained from a fungal source. For example, the enzymes may be obtained from a yeast strain such as a *Candida, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces,* or *Yarrowia* strain; or from a filamentous fungal strain such as an *Acremonium, Aspergillus, Aureobasidium, Chrysosporium, Cryptococcus, Filibasidium, Fusarium, Humicola, Magnaporthe, Monilia, Mucor, Myceliophthora, Neocallimastix, Neurospora, Paecilomyces, Penicillium, Phanerochaete, Piromyces, Schizophyllum, Sclerotium, Sporotrichum, Talaromyces, Thermoascus, Thielavia, Tolypocladium,* or *Trichoderma* strain.

In another more preferred embodiment, the enzymes are obtained from an *Aspergillus aculeatus, Aspergillus awamori, Aspergillus foetidus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Chrysosporium lignorum, Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sulphureum, Fusarium toruloseum, Fusarium trichothecioides, Fusarium venenatum, Humicola insolens, Humicola lanuginosa, Monilia sitophila, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Penicillium purpurogenum, Phanerochaete chrysporum, Polyporus pinsitus, Polyporus versicolour, Sclerotium rolfsii, Sporotrichum thermophile, Trichoderma citrinoviride, Trichoderma hamatum, Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma polysporum, Trichoderma reesei, Trichoderma saturnisporum,* or *Trichoderma viride* strain.

The enzymes may be obtained from the organism in question by any suitable technique and in particular by use of recombinant DNA techniques known in the art (c.f. Sambrook, J. et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY, USA). The use of recombinant DNA techniques generally comprises cultivation of a host cell transformed with a recombinant DNA vector, consisting of the product gene of interest inserted between an appropriate promoter and terminator, in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence may be of genomic, cDNA or synthetic origin or any mixture of these, and may be isolated or synthesized in accordance with methods known in the art. The enzyme may also be obtained from its naturally occurring source, such as a plant or organism, or relevant part thereof.

A suitable glucose oxidase (EC 1.1.3.4) may be a fungal glucose oxidase, in particular from a strain of *Aspergillus*, such as an *Aspergillus niger* glucose oxidase, or a glucose oxidase from a strain of *Cladosporium* sp. in particular from *Cladosporium oxysporum*. In the methods of the present invention, the enzymes may be obtained from commercial suppliers, preferably from Novozymes A/S. Commercially available glucose oxidases useful in the present invention are GLUZYME™ 2.500 BG, GLUZYME™ 10000 BG

and GLUZYME™ MONO 10000 BG, available from Novozymes A/S, Denmark). Other commercially available glucose oxidases useful in the present invention are FERMIZYME™ GO 10.000 and FERMIZYME™ GO 1500 available from DSM, HYDERASE™ 15 and HYDERASE™ HC available from Amano.

A suitable hexose oxidase useful in the present invention is GRINDAMYL SUREBAKE™ from Danisco A/S, Denmark.

A suitable laccase (EC 1.10.3.-) may be a fungal laccase, in particular from a strain of *Myceliopthora*. A suitable carbohydrate oxidase (EC 1.1.3.-) may be a fungal carbohydrate oxidase, in particular from a strain of *Michrodochium*, and most particularly from a strain of *Michrodochium nivale.*

A suitable catalase (EC1.11.1.6) may be a fungal glucose oxidase, in particular from a strain of *Aspergillus*, such as an *Aspergillus niger* glucose oxidase or from a strain of *Cladosporium* sp. in particular from *Cladosporium oxysporum*. The catalase may be present as a side activity of the glucose oxidase composition. In the methods of the present invention, such an enzyme composition may be obtained from commercial suppliers, preferably from Novozymes A/S. Commercially available glucose oxidases compositions comprising an appropriate catalase side activity useful in the present invention are GLUZYME™ 2.500 BG, GLUZYME™ 10000 BG and GLUZYME™ MONO 10000 BG, available from Novozymes A/S, Denmark).

The treatment of the vegetable/vegetable pieces with the one or more enzymes necessarily involves contacting the vegetable/vegetable pieces with the enzyme(s) under suitable conditions. Accordingly, the enzyme treatment may be performed by contacting the vegetable/vegetable pieces with one or more enzymes in an aqueous solution. The aqueous enzyme composition may comprise a single enzyme component, e.g., a mono-component enzyme composition, or a mixture of two or more enzymes. The enzyme treatment can be performed by immersing the vegetable/vegetable pieces in the aqueous solution. Preferably the enzyme treatment is performed by adding an enzyme preparation to the process water already applied during the process, e.g. to the rinse bath(s). In another embodiment an aqueous enzyme solution may be sprayed onto the vegetable/vegetable pieces. The enzymatic treatment of the vegetable/vegetable pieces is performed for a period of time sufficient to provide the desired property to the vegetable/vegetable pieces product. The vegetable/vegetable pieces is preferably treated for a duration of at least 1 minute, more preferably at least 2 minutes, even more preferably at least 5 minutes, and most preferably at least 10 minutes. The duration of the treatment is measured from the application of enzymes, i.e. from the beginning of submerging the vegetable in the enzyme solution or from spraying on the enzyme solution, to the inactivation of the enzyme, i.e. by boiling or heat treatment of the vegetable.

Thus, the enzymes to be used in the methods of the present invention may be in any form suitable for the use in question, e.g., in the form of a dry powder, agglomerated powder,

or granulate, in particular a non-dusting granulate, a liquid, in particular a stabilized liquid, or a protected enzyme.

In terms of enzyme activity, the appropriate dosage of a given enzyme will depend on the enzyme in question. The skilled person may determine a suitable enzyme unit dosage on the basis of methods known in the art.

In the methods of the present invention wherein the enzyme composition is added to the process water, the effective amount of the glucose oxidase, e.g. GLUZYME™ 10000 BG or GLUZYME™ MONO 10000 BG, is about 0.001 g to about 200 g enzyme protein per litre process water, more preferably about 0.01 g to about 20 g per litre process water, even more preferably about 0.1 g to about 2 g per litre process water, and most preferably about 0.2 g per litre process water.

The glucose oxidase is present in the solution in the process water in an amount effective for reducing off-flavor in the finished product, particularly 1 to 20000 GODU per litre process water, particularly 10 to 15000 GODU per litre process water, particularly 50 to 10000 GODU per litre process water, particularly 500 to 7500 GODU per litre process water or more particularly 1000 to 5000 GODU per litre process water, or most particularly around 2000 GODU per litre process water.

One GODU (Glucose Oxidase Units) is the amount of glucose oxidase enzyme, which under standard conditions (i.e. pH 5.6, 30°C, 20 min. incubation time, acetate buffer, and glucose 16.2 g/l as substrate) forms 1 micromol of hydrogen peroxide per minute.

Depending on the stability of the enzyme stability and the oxygen level in the process water the enzyme may remain active in effective amounts for 1 hr or up to several days.

The methods of the present invention may further comprise the step of blanching the vegetable. Preferably, blanching is performed prior to enzyme treatment. The blanching may be performed in accordance with procedures well-known in the art (see, for example, U.S. Patent No. 4,254,153 and Andersson et al., 1994, Critical Reviews in Food Science and Nutrition 34: 229-251). The blanching may, for example, be performed by heating the vegetable/vegetable pieces in an aqueous solution, preferably in the temperature range of about 70°C to about 100°C for about 2 to about 15 minutes, more preferably in the temperature range of about 75°C to about 90°C for about 4 to about 10 minutes, and most preferably at about 75°C for about 10 minutes. Alternatively, the vegetable/vegetable pieces may be blanched in steam, such as for about 2 to about 10 minutes.

It is understood that any of the embodiments described herein may be combined to produce a vegetable product.

The invention also relates to vegetable products obtained by the methods of the present invention.

The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

**Materials and methods**

The enzyme preparations used were commercial oxidoreductases derived from *Aspergillus niger*: GLUZYME™ 10000 BG and GLUZYME™ MONO 10000 BG both available from Novozymes A/S.

FLAVOURSTAR™, a laccase preparation comprising a laccase from *Myceliophthora* thermophila, available from Novozymes A/S.

Sensory analysis was carried out by a panel of eight judges. The flavour/taste of vacuum packaged pre-boiled potato tubers was rated using a scale from 0 to 15, where 0 referred to least off-flavour/off-taste (fresh taste) and 15 to most off-flavour/off-taste. Prior to the sensory analysis a small training was conducted comparing freshly produced potatoes (5 days of storage) with the reference applied in the evaluation (3 weeks of storage). Vacuum packed, boiled potato samples of 3 kg were out portioned in small cubs with lids and served at room temperature. The samples were tested in triplicates served in random order. The results are given as mean value of the eight judges.

**Example 1**

Glucose oxidase was tested on potato in semi-industrial scale. Potatoes (c.v. Sava, size 40/45, 20.3% DS) were de-stoned, rinsed for dirt and the peel removed by steaming and subsequently brushed off. The tubers were rinsed twice in water. 3 kg lots of rinsed potato tubers were incubated for 6 minutes at about 28°C in 3 L of enzyme solution comprising glucose oxidase (GLUZYME™ 10000 BG or GLUZYME™ MONO 10000 BG) in a concentration of 0.2 g/L and 1.6 g Dextrose/L. The water was drained and the potato tubers were vacuum-packed with approx. 100 ml of residual enzyme solution. The packed potatoes were boiled in the package for about 68 minutes at 95°C and cooled down. The vacuum-packed pre-boiled potatoes were stored for three weeks in a refrigerator at about 4°C until the sensory analysis was performed.

**Table 1: Sensory analysis of vacuum packed potatoes.**

| Treatment | Flavour/taste value |
|---|---|
| Reference | 9.95 |
| GLUZYME™ 10000 BG | 6.93 |
| GLUZYME™ MONO 10000 BG | 7.18 |

Addition of GLUZYME™ 10000 BG or GLUZYME™ MONO 10000 BG clearly reduced the amount of off-flavour/off-taste compared to the reference, and hereby improved the taste of the potatoes.

**Example 2**

A laccase preparation originating from *Myceliopthora* is tested in semi-industrial scale as described above in Example 1. 3 kg of rinsed potato tubers are incubated in 3 L of tap water containing FLAVOURSTAR™ in the amount of 1 g/L or 0.2 g/L. The improvement of taste and reduction of off-flavour note is comparable to the effect obtained with glucose oxidase in example 1.

**Example 3**

A carbohydrate oxidase from *Microdochium nivale* (*Gerlachia oryzae*) is tested in semi-industrial scale as described above in Example 1. 3 kg of rinsed potato tubers are incubated in 3 L of tap water containing 5-80 mg enzyme protein/L. The improvement of taste and reduction of off-flavour note is comparable to the effect obtained with glucose oxidase in example 1.